

# Intracellular Compartmentation of Metals in Aquatic Organisms: Roles in Mechanisms of Cell Injury

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The intracellular compartmentation of essential and toxic metals is of intense scientific interest because of its potential for adding to our understanding of both normal homeostatic mechanisms for metals and of the mechanisms which underlie metal-induced cell injury. High-affinity metal-binding proteins, lysosomes, and precipitates such as inclusion bodies or concretions, play major roles in the regulation of divalent-metal cation bioavailability. The contribution of a given compartment toward metal homeostasis is dependent upon the level exposure, cell type, organ, species, and life cycle of the organism. Toxic metals may move between these compartments, but the rates and determinants of such exchanges have not been characterized. Available data clearly indicate that sequestration of toxic metals in these specialized compartments can produce profound disturbances in the subcellular handling of essential metals. Further studies of the mechanisms by which metals partition and/or transfer among these compartments are essential to understand and predict toxicity of this important class of toxic agents.

Recent studies from a number of laboratories have identified several subcellular compartments as being major "sinks" for both essential and toxic metals in mammals and aquatic organisms. High-affinity metal-binding proteins (1-7), lysosomes (8-12), and precipitates such as inclusion bodies (13-19) or mineral concretions (7,20-27) all play important roles in intracellular metal homeostasis. The extent to which any one of these compartments is involved in metal binding appears to depend upon a number of factors, including dosing regimen for the metal administered, interactions with competing metals, cell type, organ, species, and life cycle. In addition, there appears to be movement of metals between these compartments, but the rates of metal exchange have not been determined. This review will focus on current knowledge of metal handling by these compartments, and the relationships that appear to exist between intracellular metal binding and toxicity in both mammals and nonmammalian aquatic species. This discussion will also attempt to suggest some needed areas for future research.

It is hoped that this examination will illustrate the scientific potential of the comparative approach to provide a better understanding of mechanisms of metal-induced cell injury. In particular, attention will be focused on the relationships that must exist between intracellular compartmentation of metals and their bio-

logical activity since this area appears to be of central importance to understanding mechanisms of injury under chronic exposure conditions.

## Metal-Binding Proteins

In recent years, extensive attention has been focused on the roles of soluble metal-binding proteins in the biological activity of both essential and toxic metals in mammalian (1-6,10,11) and nonmammalian (6,28-30) organisms. While a majority of these proteins appear to share similarities with mammalian metallothionein (2,31), others do not (2). A current summary of these comparative data has been recently published (2). In addition, a nomenclature system that divides SH-mediated metal-binding proteins and other low molecular weight metal-binding molecules into three (I-III) metallothionein classes based on degree of sequence homology with equine metallothionein will be published shortly (3).

Although a number of chemical differences between metal-binding proteins from various species do exist, most of these proteins appear to function as major inducible "sinks" for metals in a manner analogous to that already well known for metallothionein (1,3,6,28). Once metals enter the cell, all of these macromolecules appear to play major regulatory roles in both essential (Zn, Cu) and toxic (Cd, Cu, Hg) metal homeostasis (Fig. 1). In this regard, both mammalian and nonmammalian proteins exhibit changes in essential metal composition following toxic metal exposure (30,32). These data suggest

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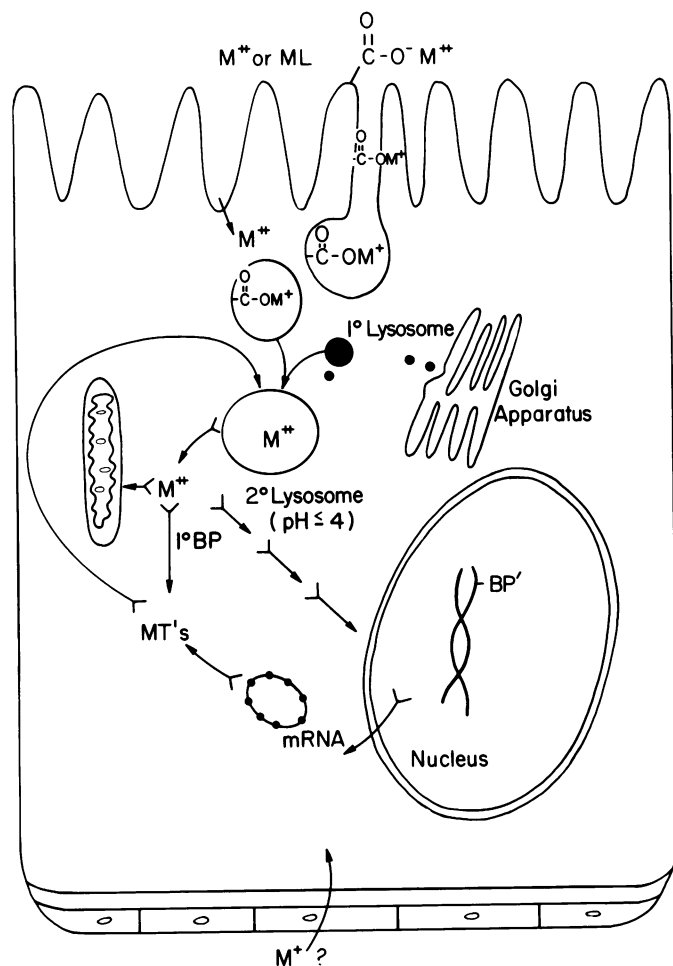


FIGURE 1. Diagram of an epithelial cell showing the induction and known and hypothesized roles of cytosolic metal-binding proteins such as metallothionein (MT) in the metabolism of metals entering the cell.

that toxic metal disruption of normal essential metal homeostasis at the molecular level may also play a role in toxicity (30) and/or repair after cell injury from these agents. In other words, sequestration of toxic metal ions

by these proteins is not without consequence with respect to the normal metabolism of essential metals in either mammalian or nonmammalian cells.

In all species studied to date, the quantity of a given toxic metal not sequestered by these metal-binding proteins (free or spill-over fraction) appears to be most closely correlated with development of cell injury (10,11,28) as defined by both ultrastructural and biochemical criteria. For example, both mammalian (10,11) and nonmammalian species (28) show increased binding of cadmium to high molecular weight cytosolic proteins once the metal-binding capacity of the inducible metallothionein or metallothioneinlike protein pools are exceeded. The implication of these data is that those toxic metal ions which are not under the homeostatic control of binding proteins actually produce toxicity when available as free cations which can react with other sensitive high or low molecular weight target molecules (5,11). At this point, it should be noted that nonmammalian organisms generally bind less (~30% versus ~90% for mammals) of their total cellular toxic metal burden to specialized soluble proteins than mammals (5,6). This may be a reflection of the generally lower dissociation constants ( $K_d$ ), e.g.,  $\sim 10^{-6}$  M for cadmium reported for nonmammalian binding proteins (6) relative to mammalian metallothionein ( $\sim 10^{-16}$  M), a lower production rate of these proteins in nonmammals and/or greater competition between intracellular compartments in nonmammals. However, nonmammalian organisms do possess several other effective intracellular mechanisms for sequestering metals which may compensate for the lower metal-binding capacity of these soluble proteins. These will be discussed below.

## Lysosomal Binding of Metals

Studies from a number of laboratories (9–12,27) have shown that lysosomes may play important roles in the intracellular bioavailability of metals in both mammalian and nonmammalian species (Fig. 1). Metals transported into the cell either bound to macromolecules or adsorbed to the cell membrane may be mobilized via proton displacement at the low pH of the lysosomes (10,27). Met-

Table 1. Subcellular distribution of  $^{109}\text{Cd}$  in rat kidney following intravenous injection of  $^{109}\text{Cd-Mt}$ .<sup>a</sup>

| Subcellular fraction | Centrifugation speed $g \times \text{min}$ | Cadmium content of fraction at various times following Cd-MT injection, % of injected dose <sup>b</sup> |                  |                  |                | Predominant organelles                    |
|----------------------|--|---|------------------|------------------|----------------|---|
|                      |  | 0.5 hr  | 3 hr             | 24 hr            | Control        |   |
| I                    | 1,500                                      | $33.2 \pm 0.6^*$  | $30.2 \pm 3.2^*$ | $22.4 \pm 2.4^*$ | $14.2 \pm 0.5$ | Nuclei, large cytoplasmic bodies          |
| II                   | 7,500                                      | $14.3 \pm 1.1^*$  | $2.7 \pm 0.3^*$  | $2.7 \pm 0.5$    | $1.4 \pm 0.3$  | Mitochondria, lysosomes                   |
| III                  | 42,800                                     | $18.3 \pm 0.8$  | $1.6 \pm 0.2$    | $1.7 \pm 0.4$    | $0.9 \pm 0.1$  | Mitochondria, lysosomes                   |
| IV                   | 292,000                                    | $6.0 \pm 0.6$   | $1.9 \pm 0.2^*$  | $1.7 \pm 0.2^*$  | $0.6 \pm 0.1$  | Mitochondria, lysosomes                   |
| V                    | 3,600,000                                  | $2.5 \pm 0.1^*$   | $1.7 \pm 0.1^*$  | $1.0 \pm 0.1$    | $0.4 \pm 0.2$  | Microsomes, small mitochondria, lysosomes |
| VI                   | 3,600,000                                  | $25.6 \pm 1.8^*$  | $61.8 \pm 5.4$   | $70.4 \pm 5.6$   | $82.5 \pm 1.4$ | Cytoplasmic sap                           |

<sup>a</sup>From Squibb et al. (10). Note that the primary lysosome fractions (II–IV) retain 2–3 times as much cadmium as the spiked control even 24 hr after Cd-MT injection when 70% of the injected dose was present in the cytosol. Rats were injected intravenously with 0.17 mg Cd/kg body weight as  $^{109}\text{Cd-Mt}$  at 0.5, 3, or 24 hr prior to sacrifice. Control rats were injected with 0.9% (w/v) NaCl and  $^{109}\text{Cd-Mt}$  was added to the kidneys at the time of homogenization.

<sup>b</sup>SEM,  $n = 3$ .

\*Indicates the number is significantly different ( $p < 0.05$ ) than the control value for the same fraction.

als released from the lysosomes to the cytoplasm in this fashion may stimulate production of metal-binding proteins as described above and/or be sequestered in precipitates such as the inclusion bodies or concretions which are discussed below. Alternatively, metal ions released within the lysosome (10) may become bound to constituents of the internal lysosomal milieu (Table 1) resulting in metal accumulation over time (Fig. 2). Lysosomes also play a role in the normal turnover of cytoplasmic proteins such as the metallothioneins (27,33,34), providing an additional means for metal accumulation in these structures.

The functional consequences of metal accumulation in lysosomes have been studied in a number of mammalian systems (11,35-37). Metals, such as mercury, may stimulate some enzyme activities while inhibiting others (35). Other metals, such as cadmium, are transported into the lysosomes bound to metallothionein. There they have been shown to disrupt the normal process of lysosomal biogenesis (11) causing functional impairment of this essential cellular system.

Finally, lysosomal autophagy of metal-damaged organelles may also cause accumulation of metals, partic-

ularly iron, in these structures. In mammals (37) this leads to marked alterations in the activities of lysosomal marker enzymes. In nonmammalian organisms (12), administration of  $\text{Hg}^{2+}$  results in iron and some mercury accumulation in lysosomes (Fig. 2), but the metabolic consequences of this phenomenon are not known. The important point here is that administration of toxic metals to mammals and nonmammals results in the lysosomal accumulation of both toxic and essential metals, suggesting another mechanism whereby toxic metals may alter essential metal metabolism.

## Intracellular Precipitates

### Intranuclear Inclusions

Morphologically distinctive intranuclear inclusion bodies have been reported in kidney proximal tubule cells following exposure to metals such as Pb (13-15), Bi (16), and Hg + Se (17). Inclusions have also been reported in hepatocytes of fish (18) following prolonged exposure to arsenic, indicating that the intranuclear inclusion phenomenon is not peculiar to mammals. Sub-

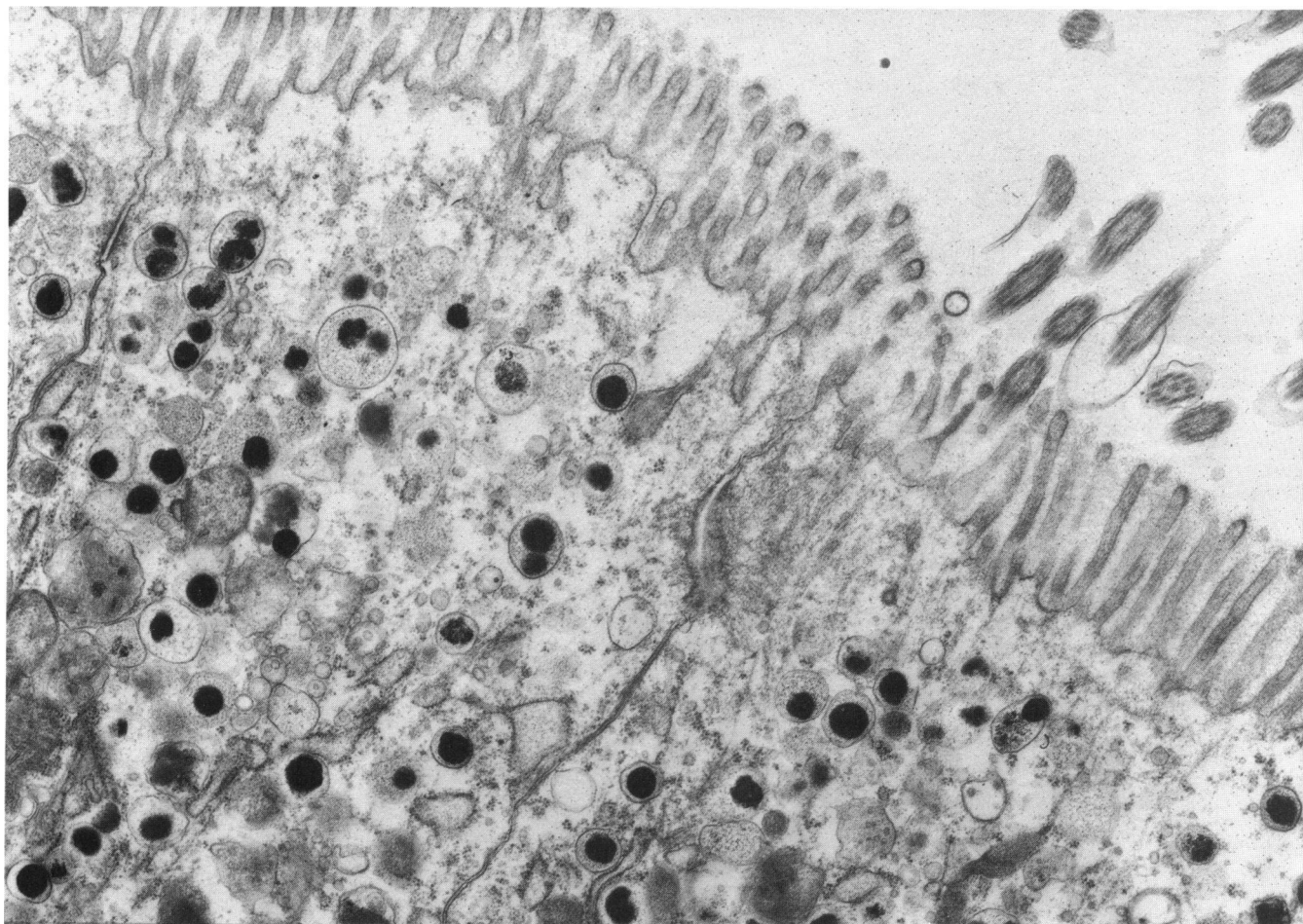


FIGURE 2. Electronmicrograph of a mantle tentacle epithelial cell showing electron dense lysosomes which were found to contain elevated concentrations of mercury and iron by X-ray microanalysis.  $\times 20,520$ .

cellular distribution studies (19) and X-ray microanalytical studies have confirmed the presence of high concentrations of these toxic metals in the inclusions. The data suggest that these structures (Fig. 3) are the major "sink" for Pb in the cell (13,19). In Pb inclusion bodies, the metal appears to be precipitated upon an inducible carboxyl-rich protein (13). The exact mechanism by which the inclusions are formed is unknown, but it appears that soluble high-affinity metal-binding proteins may be initially involved in transporting Pb into the nucleus prior to induction of inclusion body protein (38). The formation of Pb inclusions is highly susceptible to the presence of other metals such as Cd *in vivo* (39,40) and *in vitro* (41), presumably due to competition for the initial binding proteins. These data

again stress the potential importance of metal-metal interactions with regard to understanding mechanisms of intracellular bioavailability.

### Mineral Concretions

In a number of invertebrate species (7,20-27), mineral concretions (Fig. 4) composed primarily of calcium phosphate appear to be extremely important in the intracellular handling of both essential and toxic metals. These structures, which are found in large vacuoles within parenchymal cells of the kidneys (7,20-27) of molluscs, appear to be primarily involved in the regulation of calcium. They are also capable of accumulating other essential and toxic metals into the calcium phos-

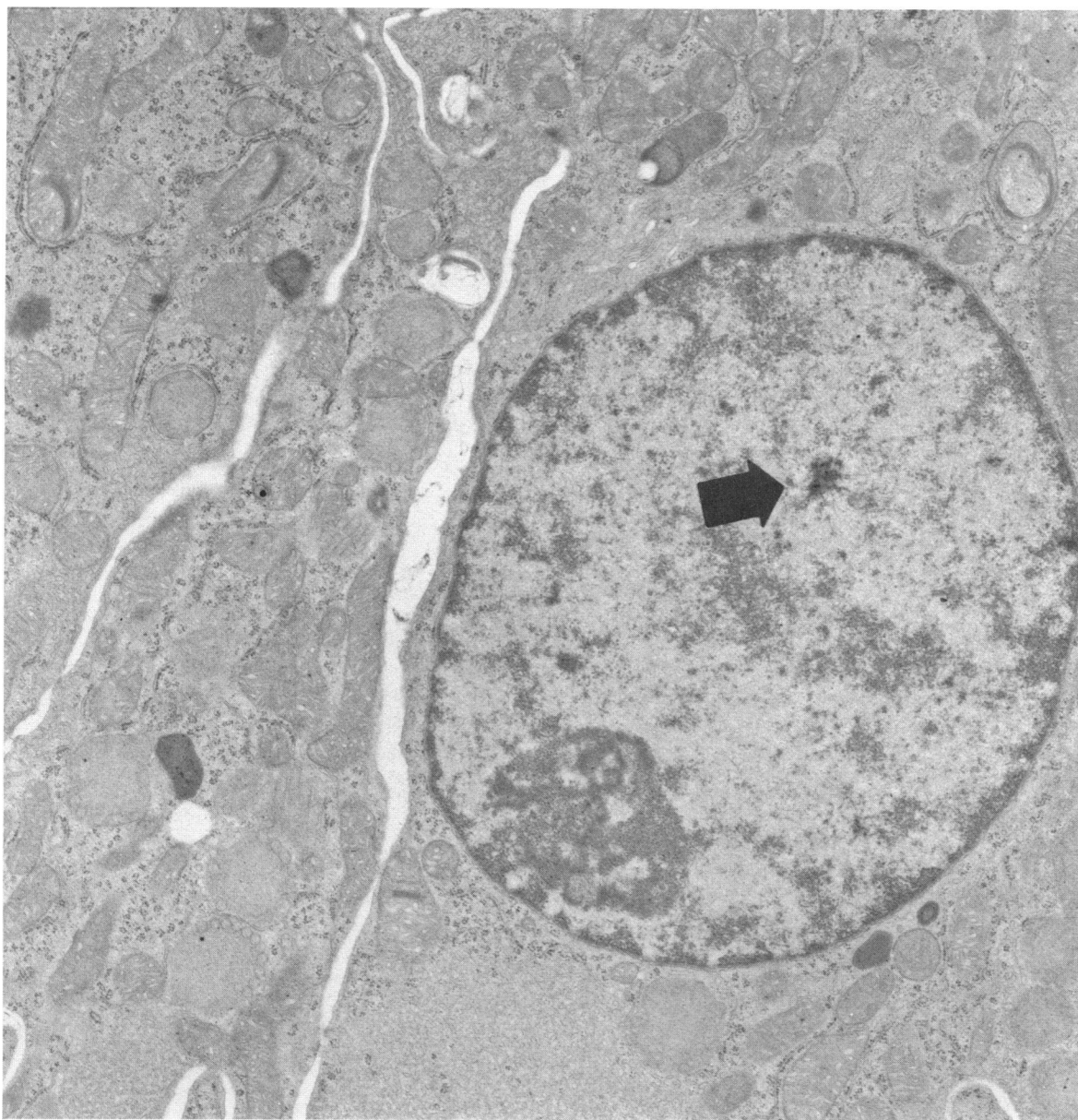


FIGURE 3. Electron micrograph of a rat kidney epithelial cell showing lead-containing intranuclear inclusion body (arrow).  $\times 14,167$ .



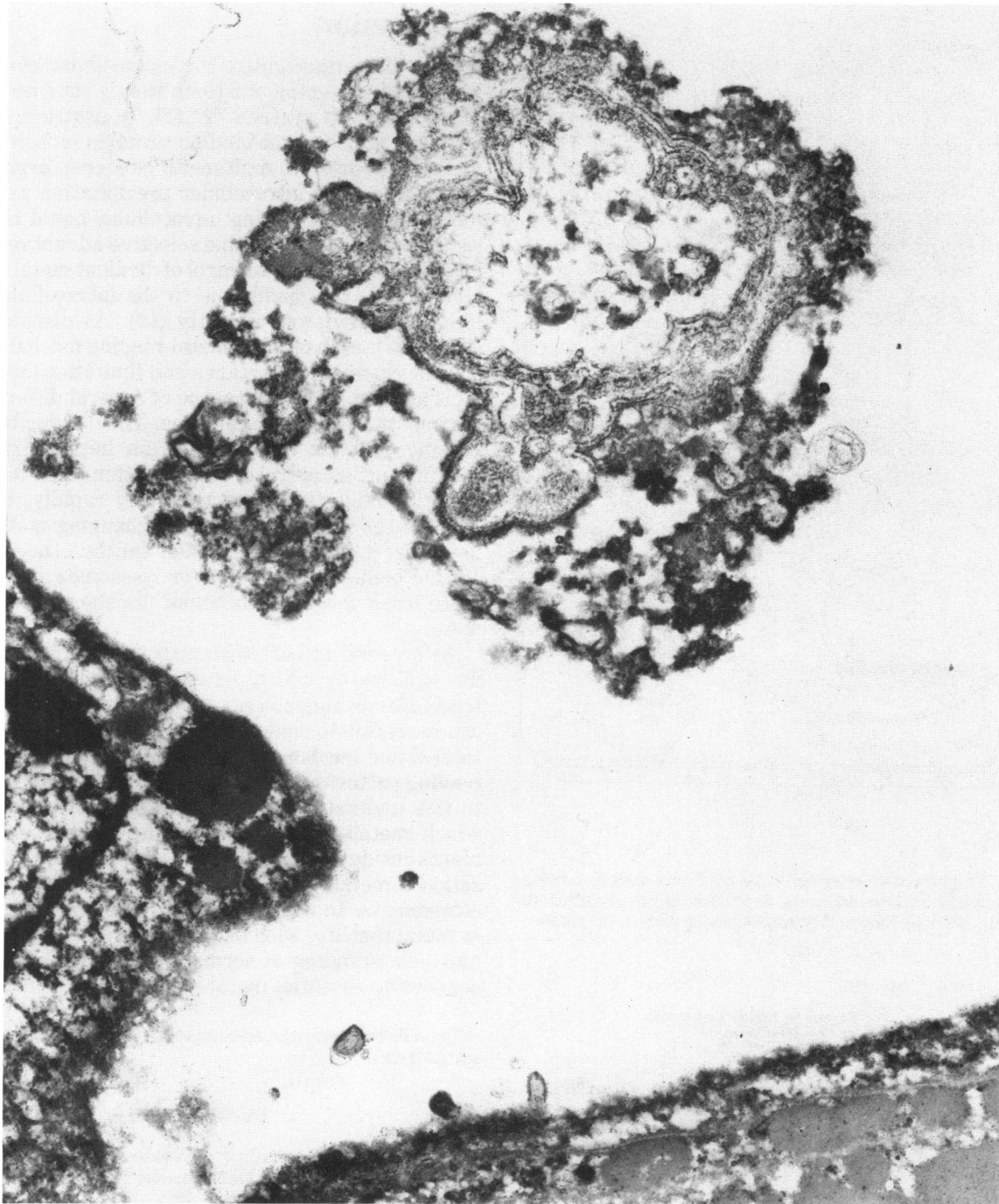


FIGURE 4. Electron micrograph of a concretion from a kidney epithelial cell of the scallop *Placopecten magellanicus* showing membranous infrastructure.  $\times 17,080$ .

phate matrix under both field (20,21,25) and laboratory (7,22-24,26) conditions. The exact mechanism of concretion formation is also not understood. However, it does appear that in some species (30) a membranous protein matrix (Fig. 5) is formed initially within a vacuole and is subsequently calcified. X-ray microanalytical studies (25,26) have shown that metal accumulation in these structures occurs at the periphery, suggesting metal deposition via accretion. Under conditions of high-

dose metal exposure (26), cells of the scallop kidney extrude concretions into the tubule lumina, with reductions in total metal renal metal concentrations (26); this suggests a depuration mechanism. The importance of the concretions in cellular handling of metals within cells containing these structures cannot be understated, but the kinetic relationships which must exist between metal binding in concretions and other intracellular compartments such as metal-binding proteins or lysosomes

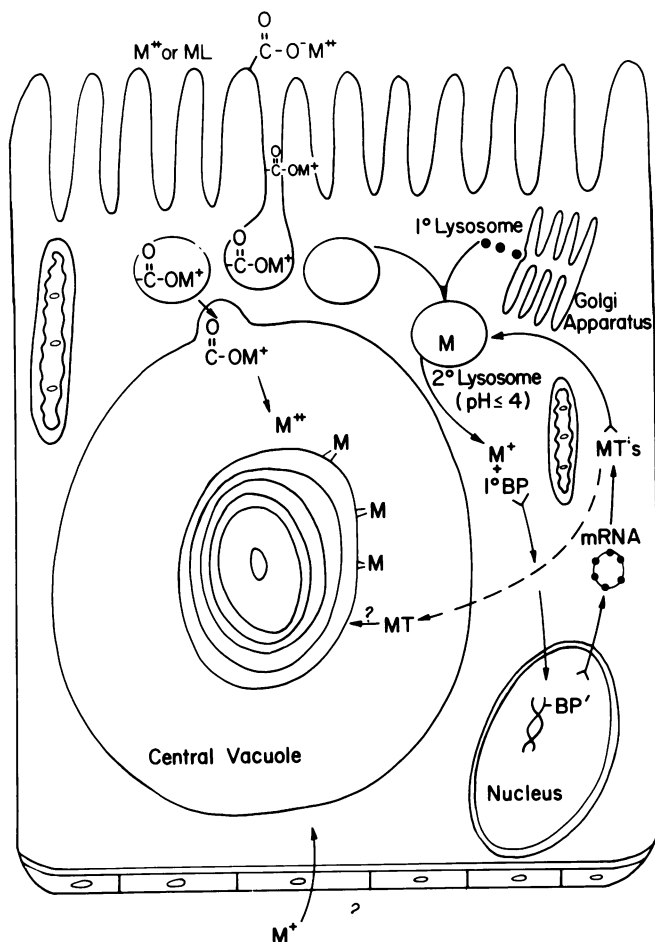


FIGURE 5. Diagram of a kidney epithelial cell from a scallop showing the hypothesized routes for metal deposition in this structure in relation to MT induction and lysosomal sequestration of metals.

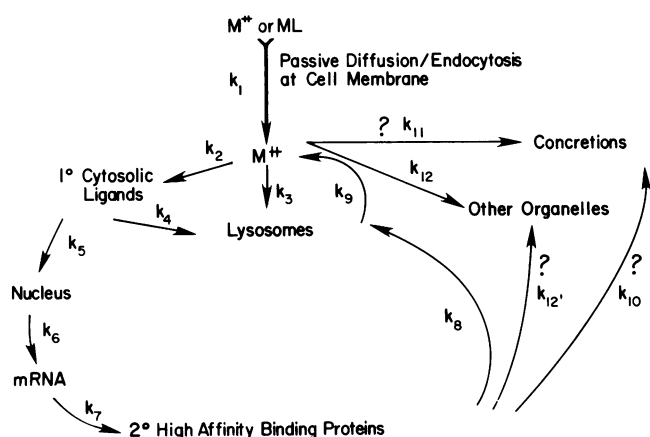


FIGURE 6. Kinetic considerations for metal partitioning between known intracellular depots for metal homeostasis.

have not been delineated. Finally, it does appear that when the concretion compartment is lost, more stress is placed upon the other intracellular "sinks," increasing the probability of toxicity (30).

## Discussion

Some bacteria regulate the intracellular bioavailability of many essential and toxic metals via specific membrane transport systems (27,42). In contrast, most eukaryotes utilize metal-binding proteins (which organize metals into discrete multimetal clusters), organelle sequestration, and intracellular precipitation as primary mechanisms of achieving intracellular metal homeostasis (27,43-49). The possible selective advantages of this change in homeostatic control of divalent metal ion availability from the membrane to the intracellular milieu have been reviewed recently (42). As discussed elsewhere (23), each of these metal-binding mechanisms has specific chemical advantages and limitations which perhaps account for the presence of several different compartments in the same cell type. Thus, inducible metal-binding proteins may provide an initial high-affinity mechanism for control of metals within the cell. Because these proteins turn over relatively rapidly, this compartment is more responsive to changing metal levels; however, it is also more labile. On the other hand, inclusion bodies, concretions, or lysosomes are probably more stable longer term "sinks" for the storage of toxic metals.

At present, in both mammals and nonmammals, the mechanisms by which metals move between these intracellular compartments are unknown, but such data are essential to understanding relationships between total tissue burden of a given toxic metal, intracellular binding patterns, and mechanisms of cell injury. Critical to this understanding is the determination of rates at which metals move between these various compartments inside the cell (Fig. 6), and a fuller characterization of metal-metal interactions within major binding components. In addition to their relevance with regard to metal toxicity, such data are extremely important to our understanding of normal physiological interactions controlling essential metal homeostasis.

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